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A BACTERIOLOGIC STUDY OF OYSTERS, WITH SPECIAL REFERENCE TO THEM AS A SOURCE OF TYPHOID INFECTION.

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A BACTERIOLOGIC STUDY OF OYSTERS, WITH SPECIAL REFERENCE TO THEM AS A SOURCE OF TYPHOID INFECTION.

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AT the suggestion of Dr. C. A. Lindsley, Secretary of the Connecticut State Board of Health, I have made some bacteriologic experiments with oysters and with the water in which they grow, in order to determine how far the bacteriologic evidence supports the idea that typhoid fever may be communicated by oysters.

The conditions favoring the communication of typhoid fever by oysters require that the Bacillus typhi abdominalis should live a few hours at least in the water in which the oysters grow, and that, having been received between the shells of the oyster, it should live in the substance or juices of the oyster until such a time as this is eaten. In this locality this time would be from a few hours to four or five days, as it is the custom to "drink" the oysters in the river after bringing them in from the sound, for a few hours or days before opening them, in order to clean them, lessen their salty taste, and fatten them.

The work of other investigators on the vitality of the B. typhi abdominalis in salt water is not quite applicable to the instance under discussion, inasmuch as the temperature of the water is not given nor the amount of salt; and indeed, in some instances, the experiments were made with a sterile salt-solution. Freytag found that the B. typhi abdominalis would live in concentrated

salt-solution five months.[1] Giaxa detected it in unsterilized sea-water after nine days from the date of infection, in sterilized water after twenty-five days.[2]

My experiments were made to find how long the B. typhi abdominalis would live in water having the same percentage of salt, the same temperature, and the same bacteria as the water in which the oysters were planted.

On December 20th a liter of water was taken from the river X, at low tide, in a sterilized flask directly over the oyster-beds. The flask was carried immediately to the laboratory, and ten c.c. of a bouillon-culture of the B. typhi abdominalis were emptied into it. The flask was then put in a wooden box outside of the window of the laboratory. Thus the temperature of the water approximated at least that of the river. The mean daily temperature of the weather during the experiment was taken from the weather-reports, and was as follows:

December 21st, 36° F.; 22d, 42° F.; 23d, 24° F.; 24th, 24° F.; 25th, 38° F.; 26th, 24° F.; 27th, 25° F.; 28th, 10° F.; 29th, 9° F.; 30th, 18° F.; 31st, 24° F.

For the first few days the water was not frozen, protected as it was in a wooden box, but during the last four days of the experiment there was a large cake of ice in the flask, and on December 30th the water was completely frozen and the flask cracked.

The last plate from the ice, made on December 31st, shows the vitality of the B. typhi abdominalis in frozen brackish water.

Agar plates were made from the flask of water on December 23d, December 27th, and December 31st. The plates were kept at 37° C. Check-plates were made also from a bottle of water from the river X, which was taken at the same time as the other sample, and was not infected with the B. typhi abdominalis. In every case

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[1] Zeitschrift für Hygiene, 1890.

[2] Zeitschrift für Hygiene, Bd. vi, p. 162.

$\frac{1}{10}$ c.c. of water was added to each plate, and in all cases in which I have stated that the B. typhi abdominalis was present cultures were made, from one or more colonies of each agar plate, on potato and in litmus milk, thus verifying the diagnosis.

The results were as follows :

Number of Colonies in $\frac{1}{10}$ c.c. of Water.

Plate of 23d, of infected water,	Innumerable cols. of B. typh. abd.
Check-plate of non-infected water,	2 or 3 cols., none resembling B. typh.
Plate of 27th, of infected water,	Innumerable cols. of B. typh. abd.
Plate of 31st, of infected water,	Innumerable cols. of B. typh. abd.

An estimation of the chlorin in the infected water was made, and there was found to be 0.1 per cent. chlorin or 0.15 per cent. salt.[1]

On January 15th two more samples of water were taken from the river X in sterilized liter flasks.[2] To one of the samples 10 c.c. of a bouillon-culture of the B. typhi abdominalis were added ; the other sample was used to make check-plates.

The flasks were kept at the temperature of the laboratory, as it was desirable to prolong the experiments, and it was feared that if exposed to the outdoor temperature the water would freeze and the flasks crack. The temperature of the laboratory during the experiment ranged from 60° F. to 70° F.

Plates were made at the dates recorded, with the following results.

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[1] For this analysis I am indebted to Prof. H. E. Smith, of the Yale Medical School.

[2] An analysis of this water showed it to contain 0.06 per cent. of salt.

Water of river X—second sample—$\frac{1}{10}$ c.c. in each plate—water infected on January 15th.

Number of Colonies in $\frac{1}{10}$ c.c.

	Water infected with B. typh. abd.	Non-infected water.
Plates of Jan. 16th,	Innumerable cols. of B. typh. abd.	4 cols ; none of B. typh. abd.
Plates of Jan. 18th,	Innumerable cols. of B. typh. abd.	4 cols.; none of B. typh. abd.
Plates of Jan. 21st,	Innumerable cols. of B. typh. abd.	5 cols.; none of B. typh. abd.
Plates of Jan. 23d,	Innumerable cols. of B. typh. abd.	2 cols.; none of B. typh. abd.
Plates of Jan. 28th,	130 cols. of B. typh. abd.	3 cols.; none of B. typh. abd.
Plates of Feb. 1st,	About 10 cols. of B. typh. abd.; many other kinds growing on plate.	Sterile.
Plates of Feb. 5th,	B. typh. abd. not discovered; plate overgrown with other kinds of bacteria.	

We may conclude from these experiments that even in extremely cold weather the B. typhi abdominalis will live in unsterilized salt or brackish water eight days at least, while in warmer water it rapidly diminishes in number after the first week, and cannot be detected in the water after three weeks.

To determine whether the B. typhi abdominalis will live and multiply in oysters, it is necessary, as a preliminary step, to find out what bacteria, if any, are found in oysters, and whether any of them resemble the B. typhi abdominalis. To determine this point some oysters were taken from the river X on November 27th, at low tide, scrubbed in city water, rinsed with sterilized water, dried on a clean towel, and opened with a sterilized knife.

The juice surrounding the oyster was drawn off with a sterilized pipet, and gelatin plates were made, each one containing one drop of the juice. Tubes of Paretti's solution were also infected with five drops each of oyster-juice. A sterilized platinum loop was plunged into the liver and stomach, twisted around, drawn out and rubbed over slant tubes of agar.

The agar tubes and Paretti's solution were incubated at 37° C., the gelatin plates at about 22° C.

The oysters used in this experiment were numbered 1, 2, 3, 4, and 5.

The results were as follows :

Tubes of Paretti's Solution.

Oyster	1	Cloudy.
"	2	"
"	3	"
"	4	"
"	5	"

Gelatin plates were made from each of the Paretti cultures, but no bacteria resembling in their growth on gelatin the B. typhi abdominalis were found. Most of the bacteria developing in Paretti's solution from oyster-juice are anaërobic and are nearly all micrococci, so that they may be readily distinguished from the B. typhi abdominalis microscopically without making cultures on gelatin.

The following is a brief description of the kinds of bacteria found in oyster-juice which grow in Paretti's solution :

(*a*) In gelatin tube-cultures this presents almost no surface-growth, but shows as a white streak along the line of puncture. There is no liquefaction, and the growth is very slow at 72° F. On potato it forms an invisible growth.

Grown in peptone-solution it gives no indol-reaction. It turns litmus milk slightly acid. It is a micrococcus,

sometimes arranged in strings like a streptococcus. It is harmless when injected into the peritoneal cavity of white rats.

(*b*) This bacterium grows dense, white, solid colonies on gelatin plates. The colonies are fairly round, but the outline somewhat dentated. After one week the colonies are nearly one-quarter of an inch in diameter. In gelatin tubes there is a thick, white, pasty surface-growth, with a good growth along the line of puncture. On potato it has a yellow growth. Under the microscope it shows large fat oval cocci.

(*c*) On gelatin plates the colonies of this bacterium are small, round and white, of slow growth. There is no liquefaction. In gelatin tube-cultures there is no surface-growth, and only a few small yellow globules along the line of puncture. There is no change in litmus milk—no indol-reaction is produced.

The microscope shows it to be a micrococcus.

(*d*) In gelatin plates the colonies after one week are minute specks. Under a one-half inch objective they appear as little, round, yellow drops.

On potato at 37° C. there is a white growth after twenty-four hours.

In gelatin tube-cultures there are little white specks along the line of puncture, with almost no superficial growth. After some days the gelatin becomes liquefied at the surface.

The microscope shows medium-sized micrococci.

Gelatin plates made with one drop of oyster-juice gave the following result:

Oyster 1. Large number of small, white colonies.
" 2. Innumerable, mostly liquefying bacteria.
" 3. Innumerable.
" 4. A few colonies.
" 5. Innumerable.

A few days later another experiment was made to determine more accurately the number of bacteria and

the kinds found in oyster-juice. The gelatin plates were made with $\frac{1}{20}$ c.c. of juice in each plate. Nine oysters were examined.

Oysters.	Number of bacteria in $^1/_{20}$ c.c. of juice.	Number of varieties noticed.	Number of liquefying in each plate.	Kinds recognized.
1	18	5	2	
2	18	5	...	Many B. fluorescens liquefaciens.
3	12	5	...	B. gasoformans.
4	39	...	3	
5	43	7	5	B. fluorescens liquefaciens.
6	48	6	4	
7	12	4	2	
8	84	10	11	
9	34	...	9	

At the same time agar plates were made with $\frac{1}{20}$ c.c. juice of the same oysters, and the plates were incubated at 37° C.

Agar Plates of Oyster-juice.

	Number of colonies in $^1/_{20}$ c.c. of juice growing at 37° C.
Oyster 1	1
" 2	3
" 3	Sterile
" 4	Many brown colonies
" 5	8
" 6	2
" 7	2
" 8	1
" 9	Many brown colonies

To determine whether the contents of the stomach contained any bacteria, the oyster was cut open with a sterilized knife and the surface of the stomach scraped

with a loop of platinum; this was then drawn over a slant tube of agar. The loop of platinum was also plunged into the liver-substance, twisted around, drawn out, and rubbed over the surface of a slant tube of agar.

Agar Tubes made by drawing a Loop of the Contents of the Stomach over the Surface of the Agar. Tubes incubated at 37° C., Bacteria in one loop growing at 37° C.

Oyster	1	Sterile.
"	2	Sterile.
"	3	Sterile.
"	4	2 cols., not B. typh. abd.
"	5	1 col., B. megaterium.

A second experiment resulted as follows:

		Bacteria in loop of stomach-contents.	Bacteria in loop of liver-substance.
Oysters	1	1 col.	Sterile
"	2	Sterile	Sterile
"	3	Sterile	Sterile
"	4	Sterile	Sterile
"	5	A few colonies	A few colonies
"	6	Sterile	Sterile
"	7	Sterile	Sterile
"	8	Sterile	Sterile
"	9	A few colonies	Sterile

From these experiments we see that the number of bacteria in one cubic centimeter of oyster-juice which grow in gelatin range from 240 to 1680. The average number is about 684.

It may be of some interest to compare the number in the oyster-juice with the number in the water taken directly over where the oysters were growing. Samples of water taken directly over the oyster-beds and secured at the same time that the oysters were taken out of the water contained on an average 9520 bacteria in a cubic centimeter. Comparing this number with the number in the

juice of the oyster we find that there are 8836 more bacteria in a cubic centimeter of the water surrounding the oyster than in the juice of the oyster. This indicates either that the juice of the oyster is destructive to certain bacteria or that the water undergoes a certain amount of filtration and purification in passing between the shells of the oyster.

The kinds of bacteria growing at 22° C. from the juice of the oyster are in most instances the same as those found in the water in which the oysters grow. Thus we find, among the more common varieties present, the B. fluorescens liquefaciens and the B. gasoformans. In one instance the B. megaterium was isolated from the stomach of an oyster. This bacterium, as is well known, is found on cabbage-leaves. There is a kind of seaweed, called by oystermen sea-cabbage, which is abundant around oyster-beds and on oysters. Whether this explains the presence of the B. megaterium in oysters I do not know.

Most of the bacteria found in the juice of the oyster do not grow at 37° C. By reference to the table of agar plates made from oyster-juice we see that there are rarely over 160 bacteria in one cubic centimeter of juice which grow at 37° C., and often there are not more than 20.

The examination of the stomach and liver of oysters shows in many cases that they are free from bacteria which grow at 37° C. The liver-substance was free in eight oysters out of nine, while the stomach was free from them in six out of nine.

I have studied chiefly the bacteria in oysters which grow at 37° C., as most growing at a lower temperature may be ruled out as a cause of disease. The bacteria from oysters growing in Paretti's solution I find, as a rule, quite different from those growing on agar plates. In the former case the bacteria are mostly anaërobic micrococci, while in the latter case they are aërobic bacilli.

Some of those growing in Paretti's solution, as already stated, resemble the B. typhi abdominalis by their invisible growth on potato, but are easily distinguished microscopically.

Two varieties isolated from agar plates liquefy gelatin rapidly, and are thus distinguished from the B. typhi abdominalis.

Three non-liquefying kinds were isolated from the agar plates, which in the early stages of growth on gelatin somewhat resembled the B. typhi abdominalis, but were easily distinguished from it by their growth on potato and in litmus milk.

Inasmuch as river-oysters, as a rule, are more exposed to contamination from sewage than oysters which are taken four or five miles away from any such source of contamination, from deep water, it seemed of interest to compare their relative infectiousness.

To do this bouillon-cultures were made by putting a half centimeter of the juice of an oyster into bouillon and allowing it to develop at a temperature of 37° C. for forty-eight hours. One cubic centimeter of this culture was then injected into the peritoneal cavity of a white rat.

Bouillon-cultures were thus made from seven river-oysters and from five oysters taken three miles out in Long Island Sound, south of the New Haven lighthouse. One cubic centimeter of each culture was then injected into the peritoneal cavity of white rats. After one month all of the five rats injected with bouillon-cultures from the sound-oysters were living and well; of the seven rats injected with bouillon-cultures of river-oysters five were living and well, but two had died.

One died three days after inoculation, the other six days after inoculation. From the blood and the liver of one of the rats a bacterium was isolated showing a white, round colony on agar plates, in litmus milk producing an intensely acid reaction, and producing a yellow slimy growth on potato.

From the blood, liver, and spleen of the other rat a bacterium was isolated, producing a brown growth on agar, with a green fluorescence. These experiments, as far as they go, seem to show a greater infectiousness of river-oysters.

It is an easy matter to determine the fact that the B. typhi abdominalis will live in oysters, but very difficult to tell whether it will propagate in them. A numerical estimation cannot be made of the number of bacteria in an oyster, even though we may know how many were introduced into it. It is clear, therefore, that conclusions in regard to number can be only very inexact.

My method consisted in cleaning a number of oysters and then injecting one cubic centimeter of a bouillon-culture of the B. typhi abdominalis with a Koch syringe between the edges of the shells. In this way a pile of oysters were infected. The oysters were kept in a cool room, the temperature ranging from 50° to 65° F. The vitality of the oysters was not apparently impaired by the operation, for, when opened subsequently, most of them appeared fresh.

Every few days one of the oysters was opened and agar plates were made from one-half a cubic centimeter of juice. The surface of the oyster over the stomach was then scorched with a red-hot iron, and the stomach was opened with a sterilized knife. A loop of the stomach contents was then drawn over a slant tube of agar. The plates and tubes were kept at 37° C.

In this way it was thought that if the B. typhi abdominalis multiplied, the agar plates and slant tubes for each succeeding oyster would show a greater number of the B. typhi abdominalis than the oysters opened before it; while if the B. abdominalis died, the juice of succeeding oysters would show a quick decrease in number.

Occasionally an oyster was opened which had lain in the same room with the other oysters, but had not been infected with the B. typhi abdominalis. Plates were

made from the juice and slant tubes from the stomach, as a check on the method.

As a preliminary experiment, some of the juice of a large oyster was drawn off with a sterile pipet, put into a sterilized test-tube, and infected with a loop of a culture of the B. typhi abdominalis.

Every few days agar plates were made with two loops of this juice. The tube of juice was kept at a temperature of between 60° F. and 70° F.

The results were as follows :

Oyster opened and juice drawn off on December 21st.

	Number of colonies in two loops of juice after infection.	Number of colonies in two loops of juice before infection
Plate of Dec. 21st . .	Innumerable colonies of B. typh. abd.	Sterile.
" Dec. 23d . .	" " " "	
" Dec. 27th . .	" " " "	
" Dec. 31st . .	About 100 cols. of B. typh. abd.	
" Jan. 10th . .	One or two cols. of B. typh. abd. ; plate overgrown with other bacteria.	

The following is the record of results with living oysters :

Oysters Injected with Bouillon-culture of B. typh. abd. on January 16th.

Oyster A. Opened January 17th.
½ c.c. juice contains innumerable cols. of B. typh. abd.
Stomach, a few cols. of B. typh. abd.

Oyster B. Opened January 19th.
½ c.c. juice contains innumerable cols. of B. typh. abd.
Stomach, 50 or 60 cols. of B. typh. abd.

Oyster C. Opened January 22d.
½ c c. juice contains 200 cols. of B. typh. abd.
Stomach, 2 or 3 cols. of B. typh. abd.

Oyster D. Opened January 24th.

½ c.c. juice contains innumerable cols. of B. typh. abd.

Stomach, about 14 cols. of B. typh. abd.

Oyster E. Opened January 28th.

½ c.c. juice contains innumerable cols. of B. typh. abd.

Stomach, sterile.

Oyster F. Opened January 28th. Check from pile not inoculated with B. typh. abd.

½ c.c. juice, sterile.

Stomach, sterile.

Oyster G. Opened February 2d.

½ c.c. juice contains about 60 colonies of B. typh. abd.

Stomach, sterile.

Oyster H. Opened February 13th.

½ c.c. juice contains about 40 cols. of B. typh. abd.

Stomach, 3 cols. of B. typh. abd.

In stating the number of colonies of the B. typhi abdominalis an exact number cannot be given, as there may have been in some instances other kinds of bacteria present which were mistaken for the B. typhi abdominalis. In general, however, the number is approximately correct, as cultures were made from some of the colonies of every plate, in litmus milk and on potato, and the mere plate appearance was not alone relied upon to determine whether I was dealing with the B. typhi abdominalis or some interloper.

These experiments do not throw very much light on the question of the multiplication of the B. typhi abdominalis in oysters. They do, however, seem to show that if multiplication does occur it takes place within the first two weeks, and that after that there is a progressive decrease in the number of the B. typhi abdominalis found in oysters, but that it may be found even after thirty days from the date of infection. They further show that the B. typhi abdominalis not only lives in the juice, but penetrates into the stomach and lives there for some time. In fact the B. typhi abdominalis lives longer in the juice and stomach of the oyster than it does in the water in which the oyster grows.